IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of
DONALD S. ANSON ET AL.

Attorney Ref: 604-56

Serial Number:

Group Art Unit: 184

Filed: August 12, 1988

Examiner: D. Crouch

For: FACTOR IX PROTEIN

DECLARATION UNDER RULE 132

PROFESSOR GEORGE GOW BROWNLEE declares as follows:

1. I am one of the inventors of the above-identified patent application, together with Dr. Donald S. Anson and Dr. Ian M. Jones and one of the co-authors of the paper by Anson et al., referenced at page 10 lines 23, 30 and 35 of the patent application text.

2. I have read the official action dated 17th June 1991 in respect of this application and I comment upon page 2 lines 12 to 19 as follows.

3. As stated at page 10 line 34 to page 11 line 2 of the patent application text, the starting factor IX DNA of the examples was the cDNA clone cVI described in Figure 1 of the Anson et al. paper. That clone cVI lacked nucleotides 1 to 24 of the mRNA and had nucleotides 25 to 39 in inverted and complementary order. Steps were attempted (described at page 11 lines 3 to 34 of the patent application text) to correct the nucleotide sequence and give a recombinant designated p5'G/3'cVI.

4. After the patent application had been filed it came to my attention that page 20 lines 21 to 28 of the patent application text was in error in suggesting that p5'G/3'cVI DNA was used to prepare deposited plasmid pIJ5/9. It was in fact the original clone cVI DNA which was incorporated in plasmid pIJ5/9. Thus deposited plasmid also lacks nucleotides 1 to 24 and has nucleotides 25 to 39 in inverted and complementary order. However, despite this, the plasmid led to the production of fully active factor IX protein.

5. I declare further that all statements made herein of my own knowledge are true and that all statements made on information are belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issued thereon.

9th Aug 1991

Date

G. G. Brownlee

George Gow Brownlee

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The EMBO Journal vol.3 no.5 pp.1053–1060, 1984

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The gene structure of human anti-haemophilic factor IX

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Communicated by G.G. Brownlee

The mRNA sequence of the human intrinsic clotting factor IX (Christmas factor) has been completed and is 2802 residues long, including a 29 residue long 5′ non-coding and a 1390 residue long 3′ non-coding region, but excluding the poly(A) tail. The factor IX gene is ~34 kb long and we define, by the sequencing of 5280 residues, the presumed promoter region, all eight exons, and some intron and flanking sequence. Introns account for 92% of the gene length and the longest is estimated to be 10 100 residues. Exons conform roughly to previously designated protein regions, but the catalytic region of the protein is coded by two separate exons. This differs from the arrangement in the other characterized serine protease genes which are further subdivided in this region.
Key words: Christmas disease/clotting factor IX/gene cloning/haemophilia B/mRNA

Introduction

Factor IX (Christmas factor) is the precursor of a serine protease required for blood clotting by the intrinsic clotting pathway. Clinically, defects in this factor result in haemophilia B (or Christmas disease), and this X-linked disorder occurs in ~1 in 30 000 males (reviewed by McKee, 1983). Patients are treated with factor IX prepared from pooled plasma from normal individuals.

Cloning of the mRNA and the gene for factor IX from normal human sources is a necessary preliminary to a number of future studies, some of direct clinical relevance to haemophiliacs and their families, and some of academic interest. The first clones isolated by ourselves from part of the human gene (Choo *et al.*, 1982) have already proved useful in demonstrating extensive gene deletions in one subgroup of patients (Giannelli *et al.*, 1983), although this study was limited by a lack of 'probes' covering the entire factor IX gene. Clones covering the coding region of the human factor IX mRNA (Kurachi and Davie, 1982; Jaye *et al.* 1983) have been used to demonstrate a naturally occurring frequent *TaqI* polymorphism (Camerino *et al.*, 1984). Clones have now been successfully used for carrier diagnosis in several Christmas disease families (Giannelli *et al.*, 1984; Peake *et al.*, 1984). Probes have also been used to localize the factor IX gene to the Xq2.7 region of the X chromosome (Boyd *et al.* 1984; Camerino *et al.*, 1984).

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We report here the complete nucleotide sequence of the factor IX mRNA and an extensive characterization of the gene defining the promoter region, the mRNA start, the eight exon regions and the mRNA stop point. This should provide a more secure foundation for further studies of the molecular pathology of the disease and for the isolation of further polymorphisms for use in diagnosis, as well as provide a basis for studies of the expression of factor IX protein from recombinant DNA sources. If successful, this would prevent the risks of hepatitis or acquired immune deficiency syndrome (AIDS) present in the current treatment of haemophiliacs.

Results

cDNA cloning and sequence analysis of factor IX mRNA

Bovine factor IX mRNA is enriched in the 20–22S fraction of liver mRNA (Choo *et al.*, 1982). Assuming human mRNA to be similar, we constructed cDNA libraries from this same sized fraction of a normal human liver (see Materials and methods). Factor IX clones were identified using a previously isolated exon probe (Choo *et al.*, 1982) and four overlapping clones were characterized and used to derive the sequence of the factor IX mRNA (see Materials and methods and Figure 1). Clone cVII was the longest cDNA clone which was fully characterized and it extended from residues 41 to 2026 of the mRNA sequence (see Figure 2). However, the sequence between residues 41 and 135 was inverted and complementary in sense with respect to the remaining sequence. Clone cVI was also rearranged in a similar manner in its first 15 residues. Both rearrangements are presumably due to cloning artefacts. Clones 108.1 and DB.1 provided overlapping sequences to complete the 3′ non-coding sequence and to define the location of the poly(A) tail.

Figure 2 shows the mRNA sequence derived from these cDNA clones and this includes evidence on the sequence of the 5′ non-coding region and the mRNA start point derived subsequently from the analysis of genomic clones (see below). The mRNA is 2802 residues long; it contains a short 29 residue long 5′ non-coding sequence and an extensive 1390 residue long 3′ non-coding sequence including the UAAUGA

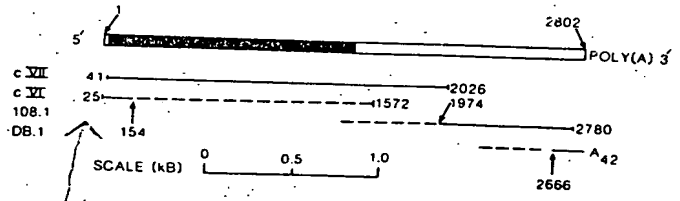

Fig. 1. Line diagram of four overlapping cDNA clones used in the sequence analysis of the mRNA. The block diagram represents the structure of the factor IX mRNA. The solid area represents coding and the open areas 5′ and 3′ non-coding sequence. The four clones and their identification symbols are shown, with solid lines representing sequenced and dashed lines unsequenced regions. The extent of the sequenced regions and of the clone (if known) is indicated by the nucleotide number (see Figure 2). Clone cVII was previously referred to as probe V (Giannelli *et al.* 1983).

NB

NB cVI starts at 25; therefore 1 to 24 was the region we originally intended to correct